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BACTERICIDAL ACTIVITY OF THE BLOOD OF PERSONS VACCINATED
WITH VARIOUS TYPHOID AND TYPHOID-PARATYPHOID VACCINES

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With Various Typhoid and Typhoid-Paratyphoid Vaccines

by

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13. ABSTRACT <p>Changes in various indices of the blood bactericidal reaction were studied in persons immunized with typhoid and typhoid-paratyphoid vaccines of known epidemiological efficacy. No correlation was revealed between the efficacy of the preparations and their capacity to increase the phagocytic activity of leukocytes to O- and Vi-strains of typhoid bacilli, to stimulate the thermostable and thermolabile bactericidal activity of the blood, or to effect the general complementary activity and properdin system indices. Following vaccination, elevation of blood phagocytic activity and properdin content was observed in persons with relatively low indices before immunization, while no corresponding changes were detected in persons with relatively high initial values.</p>		

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BACTERICIDAL ACTIVITY OF THE BLOOD OF PERSONS VACCINATED WITH VARIOUS TYPHOID AND TYPHOID-PARATYPHOID VACCINES

In recent years there has been growing interest in studying the dynamics and mechanisms of the effect of humoral and cellular protective factors, participating in the process of ridding the organism of agents of infection (Adlar, 1953; Land' and Pillemer, 1956; Mushel and Treffers, 1956; Soulier and Menache, 1958; Delaknay, 1958; Isliker, 1959; Bagzasarov et al., 1961; Khazanova, 1964; Suponitskaya, 1964, 1965; Martynova and Bissarionova, 1965, etc.). Testing was undertaken to evaluate the intensity of post-vaccinal immunity against typhoid fever. According to the activity of one of the indices of bactericidal reaction of blood to typhoid microbes (Melikova et al., 1953; Melikova, 1963; Karolchek et al., 1961, 1962; Ladosz, 1964 etc.). We have shown in previous research (Levina et al., 1965) that vaccination against typhoid fever leads to a brief increase in the content of properdin and the third (C'3) component of the complement, without causing changes in the content of other components and the overall complementary activity. In vaccinated people there was an increase in phagocytic activity of the leukocytes in relation both to homologous (typhoid fever) and heterologous microbes (staphylococcus).

The purpose of our work was comparative study of the dynamics of various indices of bactericidal activity in the blood of persons vaccinated with typhoid fever and typhoid-paratyphoid B vaccines with various epidemiological efficacy.

In the works heated, alcoholic, and chemical vaccines were used, which were tested in the USSR in epidemiological experiments in 1961 and 1962 (Kheysets et al, 1964, 1965), and also vaccines K-VOZ and L-VOZ, which are undergoing study in Guiana and Yugoslavia (Yugoslav Typhoid Commission, 1964). Preparations were injected subcutaneously to volunteers not previously vaccinated (16-50 years in age) twice at 20-30 day intervals.

The phagocytosis reaction was carried out according to the generally accepted method, using a suspension of live 20-hour agar culture of *S. typhi* 4446 (Vi-strain) and typhoid O-diagnostic (receptor IX).

The bactericidal activity of the blood was determined by two methods, the first, using an exogenous complement. For this purpose, whole serum was heated at 56° for thirty minutes, and 5-fold dilution of it was prepared (1:5-1:3125), then about 0.5 ml of each dilution of the serum was poured into test tubes, 0.5 ml of the 20-hour broth of a culture of Vi-strain of typhoid bacteria, diluted 1:100,000, and 0.5 ml of guinea pig serum at a dilution which did not show a bactericidal effect. In control test tubes, in place of serum or complement, 0.5 ml of physiologic solution at pH 7.0 was added. The test tubes were vibrated and mixed in a water bath at 37° for two hours, and then 0.1 ml of the contents of each test tube was inoculated in two plates with weakly alkaline agar. Analysis of the colonies was carried out after 24 hours of incubation of the plates at 37°. Serum was considered to be active in which not more than 50% of the colonies grew in comparison to the control.

Secondly, the bactericidal activity of blood was established using endogenous complement. For this method, to 0.4 ml of native serum (fresh or kept at -70°) were added 0.1 ml of a suspension (veronal buffer pH 7.2) of 20-hour agar culture of Vi-strain of typhoid bacteria, containing 200 million microbial cells per ml. The mixture was carefully vibrated and mixed in a water bath at 37° for two hours. Then the contents of the test tubes were refrigerated at 0° in an ice bath (with the purpose of curtailing the bactericidal reaction) in 5 serial 5-fold dilutions carried out; 0.1 ml of the mixture of each dilution was seeded in a plate with weakly alkaline agar. In a controlled test tube in place of serum, 0.4 ml of veronal buffer was added. Analysis of colonies was

carried out after incubation of the plates in a thermostat at 37° for two days. The activity was expressed in the form of a bactericidal index (Karolcek et al, 1961, 1962);

$$\text{BI-log } \frac{\text{number of live microbes in control}}{\text{number of live microbes in experiment}}.$$

Properdin was determined with the help of a variation of the zymozan method, proposed by Mashkov and Mikhaylova (1962). The properdin content increased in per cent of an activated guinea pig complement (RP), added in the presence of zymozan to 0.1 ml of the test serum (fresh or stored at -70°). The complement and its components were determined by complete hemolysis by titration with preserved reagents according to the method of Mashkov et al (1963)¹. Moreover, the complement content was established by titration of native serum, using a 7% suspension of sensitized sheep in physiologic solution.

The presence of so-called semiquantitative value during the determination of properdin, complement and its components required the use of the median as the average value. The confidence intervals of the median were determined according to the table of Ashmarin and Vorob'yev (1962). Statistical analysis of research materials was carried out at a level of significance of 0.05.

Research materials indicated that the dynamics of various indices of bactericidal activity depended on the initial value (Table 1). Thus, in our observations the data of Karolcek et al (1961) on the absence of an increase of bactericidal activity upon vaccination of persons with an initial bactericidal index equal to two or greater, were confirmed. Moreover, it was established that in persons with initial indices of phagocytosis exceeding 40%, as in persons whose serum inactivated more than 20% RP before vaccination, an increase in these indices after vaccination was not revealed.

In studying the dynamics of phagocytic activity in persons vaccinated (the increase in phagocytic activity in vaccinated persons) the increase in phagocytic activity was determined in relation to O- and Vi-strains of typhoid

¹ Properdin and components of the complement were determined in the microbiological laboratory of the Institute of Pediatrics AMN SSSR in collaboration with Z.M. Mikhaylova.

microbes) with various intensities of phagocytosis, vaccinated with preparations of various epidemiological efficacy (for example, the more effective alcoholic and less effective chemical vaccine of the Moscow Institute of Vaccines and serum named for Mechnikov), were not revealed (Table 2). A difference in the intensity of phagocytosis was not found in persons vaccinated with highly effective heated vaccine and typhoid-paratyphoid B divaccine of the Institute of Epidemiology and Microbiology named for Gamalei - with a preparation which did not protect people from typhoid fever in the epidemiological experiment of 1962. Similar results were obtained by Ladosz (1964), who studied the opsonization effect of serum from persons immunized with vaccines tested in Poland in the immunological experiment of 1961.

Table 1

Dependence of Dynamics of Indices of Immunity in Persons On Initial Value

Index Determined	Initial index according to examined group	Number before vaccination	Activity at various period after first vaccination		
			20-30 days	30 days	30 days
Бактерицидная активность —	2 and more	5 49	2.67 (0.09) —	2.58 (0.04) 2.79 (0.17)	2.58 (0.13) 2.74 (0.19)
Средний индекс и его стандартная ошибка (в ед.)	more than 20 and less	47 44 66	10 (<10-10) — 40 (30-40)	30 (25-30) 10 (<10-10) 30 (25-40)	30 (25-40) 30 (25-40) 40 (30-40)
Пропердин — медиана и стандартный интервал (в %)	less than 20	70 141	— 20.7 (0.9)	30 (25-40) 45.7 (2.4)	40 (30-40) 56.3 (2.5)
Фагоцитоз — средняя арифметическая и ее стандартная ошибка (в %)	more than 40	79 47 55	— 60.3 (2.1) —	24.1 (1.2) 48.3 (3.3) 69.1 (1.8)	47.3 (3.0) 45.9 (3.4) 58.9 (3)

F=Bactericidal activity - average index and its standard deviation (in units)

G=Properdin - median in activated RP and its confidence interval (int)

H=Phagocytosis - arithmetic mean and its standard deviation (in %)

Note. Here and in Table 2 in brackets the first number is the standard deviation of the index, the second is the confidence interval.

Table 2

Dynamics of Phagocytic Activity of Human Blood

Preparation for vaccination	Test Microbe	Vaccine	Activity at various times examined			Activity at various times examined				
			F	G	H	F	G	H		
Typhoid Monovaccine	O-typoid mono-diagnostic IX receptor	Heated 1961	13 (3.3)	21.5 (4.9)	28.4 (6)	64.6 (3.1)	12 (2.4)	19.3 (7.2)	28.6 (5.3)	67.7 (5.7)
		Alcoholic 1961	14 (2.4)	23 (7.2)	57.5 (3.5)	71.6 (2.4)	13 (2.3)	23 (8.3)	57.5 (7.1)	71.6 (3.5)
		Chemical of the Inst. of Vaccines & Sera named for Technikov	14 (1.5)	20.2 (7.9)	56.0 (7.7)	47 (7.7)	13 (2.3)	17.3 (8.3)	54.7 (7.1)	42.9 (7.1)
	20-hour culture of S. typhi 4445	Acetone K-voz L-voz Heated 1963	19 (5.4)	47.2 (4.9)	52.5 (2.9)	46.4 (7.5)	8 (2.9)	25.2 (2.9)	44.5 (7.3)	47.3 (7.7)
Typhoid Paratyphoid divaccine	20-hour agar	Heated 1962	18 (2.1)	22.1 (5.4)	40.2 (5)	41.1 (5)	17 (2.5)	20.9 (2.5)	40 (5.8)	41.5 (5.4)
	culture of S. typhi 4446	Alcoholic 1962	27 (4.7)	32.5 (5.6)	45.1 (5.9)	60.2 (5.9)	19 (2.7)	19.5 (7.5)	49 (7.6)	63.4 (7.6)
		Chemical of the Inst. of Vaccines & Sera named for Technikov 1962	18 (4.9)	24.3 (6.9)	45.2 (7.2)	49.6 (7.2)	14 (2.7)	14.9 (2.7)	45.4 (8.2)	55 (7.6)
		Chemical of the Inst. of Epidemiology Microbiology named for Gamalei 1962	29 (4.2)	33.1 (5.1)	38 (6.3)	56.9 (6.3)	20 (3.1)	21.4 (6.8)	43.2 (7.7)	58.6 (7.7)

F=Before vaccination

G=20 - 30 days after first vaccination

H=30 days after second vaccination

~~Arithmetic mean and its standard deviation.~~

The effect of vaccination on bacteriolytic activity of human serum was not successfully revealed (Table 3). Attention was turned to the large number of sera not possessing activity among the volunteers examined in comparison with sera of persons ill with typhoid fever, confirmed by isolation of hemoculture. Thus, in the group of healthy persons, bacteriolytic activity was not detected in 35 out of 99, and in the group of typhoid fever patients - in one out of 21.

Table 3

Bacteriolytic Activity of Sera from Persons
Vaccinated with Typhoid and Typhoid-
Paratyphoid B Vaccines (Test culture -
Vi-Strains of S. Typhi)

Vaccine	Number examined	Number of sera from persons inoculated with vaccines			
		Typhoid	Para-	Typhoid	B
		F	G	F	G
Acetone K-voz	15	9	5	6	5
Heated L-voz	18	9	1	9	7
Heated 1962	16	8	1	8	6
Alcoholic 1962	15	7	2	8	7
Chemical of the Institute of Vaccines and Sera named for Mechnikov 1962	15	8	4	7	5
Chemical of the Institute of Epidemiology and Microbiology named for Galmalei 1962	15	6	2	9	
Total....	94	47	15	47	40

F=Inactive before Vaccination

G=With activity developed after Vaccination

While studying the bactericidal activity of sera with the use of endogenous complement it was noted that a large number of persons not suffering from typhoid fever and not vaccinated had relatively high bactericidal indices (at least 2) in relation to 3 Vi-strains of typhoid microbes studied (Bacnagar Vi-1 4446 and Ty₂). Because of the low number of sera with relatively low bactericidal activity before vaccination we did not succeed in comparing this index in volunteers vaccinated with various vaccines. At the same time, differences were not detected in bactericidal activity in persons with relatively high initial indices, vaccination with effective and ineffective vaccines (Table 4). Differences in the dynamics of bactericidal activity of sera in relation to three tested Vi-strains of typhoid fever microbes were not detected either. Moreover, the highest bactericidal activity was determined in relation to the Bacnagar strains in the lowest, in relation to the Ty₂ strain.

Table 4

Bactericidal Activity in Relation to Strain S. typhi in Blood of Persons Vaccinated Against Typhoid Fever

Test Microbes	Vaccine	Mean bactericidal index	Activity at Various times ¹		
			E	F	G
Vi-strain of S. typhi	Heated 1963	11	2.67 (0.14)	2.75 (0.14)	2.54 (0.25)
	Alcoholic 1962	11	2.67 (0.13)	2.48 (0.18)	2.73 (0.13)
	Chemical from Inst. of Epidemi- ology & Microbi- ology named for Galmalei	12	2.75 (0.11)	2.65 (0.16)	2.50 (0.19)
	Bacnagar Vi-1 S. typhi 4446	12	3.2 (0.06)	3.31 (0.09)	3.21 (0.13)
	Typhoid and Para		2.82 (0.13)	3.03 (0.14)	2.95 (0.18)
	Typhoid B vaccine		2.15 (0.12)	1.95 (0.04)	2.18 (0.14)

E, Before Vaccination F, 20-30 days after 1st vaccination
 G, 30 days after second vaccination

I. Bactericidal Index and its standard deviation (in units).

An increase in the properdin level was noted in vaccinated persons in all groups (Table 5). However, the increase proved to be substantial only upon overall analysis of these examinations in groups of persons immunized with acetone vaccine K-voz. The mean index of properdin in this group was not substantially different from indices determined in all other groups of vaccinated volunteers.

No substantial changes in the overall complementary activity or in the content of components of the complement were detected in persons vaccinated with all preparations studied.

Thus, after immunization within intestinal vaccines the indices of bactericidal reaction studied in the blood of persons with relatively low initial value increased. No differences in the dynamics of the indices in persons vaccinated with typhoid mono- and typhoid-paratyphoid B vaccines prepared by various methods and of varying epidemiological efficacy were noted.

We did not intend the fundamental material for analysis of the role of bactericidal activity of blood in human immunity against typhoid fever. Nevertheless, the results of our research permit the assumption that the intensity of postvaccinal immunity cannot be determined on the basis of indices of the bactericidal reaction. Thus, the blood of many volunteers, not previously vaccinated, possess high bactericidal activity. Changes were not revealed in the bactericidal activity in persons with high initial indices after vaccination either. Finally, the injection of the chemical divaccine of the Gamalei Institute of Epidemiology and Microbiology which was not effective in the epidemiological experiment produced an increase in phagocytic activity of leucocytes with the same intensity as did injection of highly effective heated vaccines.

CONCLUSIONS

1. The injection of typhoid fever and typhoid-paratyphoid B vaccines produced an increase in the properdin level and phagocytic activity of leucocytes in the blood of persons with relatively low initial indices. In persons

Table 5

Properdin Index in Persons Vaccinated with Various Typhoid Fever and Typhoid-Paratyphoid B Vaccines

Vaccine	Number examined	Activity at various times ¹				
		a Before Vaccin- ation	b	c	d Before Vaccin- ation	e
Acetone K-VOZ	17	20 (<10—30)	30 (20—30)	50 (40—50)	5 (<10—20)	30 (10—30) 40 (20—50)
Heated L-VOZ	21	20 <td>30 (20—40)</td> <td>40 (25—50)</td> <td>12 (<10—10)</td> <td>30 (10—40) 30 (10—50)</td>	30 (20—40)	40 (25—50)	12 (<10—10)	30 (10—40) 30 (10—50)
Heated 1962	15	30 (10—40)	30 <td>30 (10—40)</td> <td>7 (<10—20)</td> <td>30 (10—40) 30 (10—40)</td>	30 (10—40)	7 (<10—20)	30 (10—40) 30 (10—40)
Alcoholic 1962	25	30 (20—40)	30 (10—40)	30 (20—40)	10 (<10—20)	10 (10—40) 15 (10—30) 35 (<10—40)
Chemical Institute of Vaccines & Serums named for Machnikov 1962	14	30 (10—40)	20 (10—30)	30 (25—40)	5 (<10—20)	10 (<10—20) 10 (<10—30) 40 (<10—40)
Chemical Institute of Epidemiology & Microbiology named for Gal'mai 1962	26	30 (10—40)	30 (10—40)	40 (30—40)	10 (<10—20)	10 (<10—30) 30 (<10—40)

1. Median and its confidence interval (in %)

a, d, E=20—30 days after 1st vaccination

b, e, F=30 days after second vaccination

— Number examined with index of 20% and less

with high initial indices the corresponding changes after vaccination were not detected. Therefore, analysis of immunological displacements after vaccination of persons with relatively low and high initial indices should be carried out separately.

2. The correlation between epidemiological efficacy of typhoid fever vaccine and their capacity to stimulate bactericidal activity of blood was not established.

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